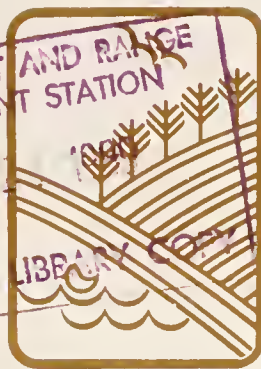


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USDA Forest Service

Rocky Mountain Forest and  
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## Number of Fungal Colonies Required to Describe Species Differences on Reclaimed Coal Mine Areas in New Mexico

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A minimum of 189 soil fungal colonies growing on rose bengal-streptomycin agar plates are required to describe species differences (diversity) in the fungal community from soil collected from an undisturbed pinyon/juniper plant community within the McKinley coal mine in west-central New Mexico.

**Keywords:** richness, density, diversity, Shannon's index, fungal ecology

Currently, the diversity of plant species must be evaluated on revegetated coal mined lands prior to bond release (Aldon 1984, Chambers 1983). The stage in succession to a stable soil ecosystem may also be evaluated by microbial diversity indices (Atlas and Bartha 1981). Several investigators, for example, have used microfungus population, community composition, and diversity responses to gain a better understanding of ecosystem functioning and development on disturbed and reclaimed areas (Fresquez et al. 1986, Lawrey 1977).

Species diversity indices, such as Shannon's diversity index, relate the total number of species in the community (richness) with the total number of organisms counted (density) (Zar 1974). Prior to the estimation of microbial diversity, however, a species area curve should be generated (Allen 1985). The objective of this study was to determine the minimum number of fungal colonies that would have to be isolated on rose bengal-streptomycin agar plates in order to describe species differences in the soil fungal community from soil collected from a pinyon/juniper plant community.

### Materials and Methods

A composited soil sample (10 subsamples) was obtained from an undisturbed pinyon/juniper plant community located within the McKinley Coal Mine in

west-central New Mexico. Each soil subsample was collected randomly to a depth of 13 cm with a 5-cm-diameter bucket auger, composited in a sterile plastic container, placed in an ice-chest, and transported back to the laboratory where the soils were passed through a 2-mm sieve.

Inoculation of a set of plates (5, 10, 15, 20, and 25) included placing 1 mL of a  $10^{-3}$  dilution from a 10-g oven-dry weight equivalent soil sample in a petri dish, adding cooled rose bengal-streptomycin agar (Martin 1950), swirling for an even distribution, and incubating at 27°C for 7 days. Each set of plates was replicated three times.

After incubation, a portion of every colony was transferred to a carrot agar medium by removing a portion of the agar containing hyphal tips. After a 4-day incubation period, the colonies were identified and tabulated using the taxonomic guides of Barnett and Hunter (1972), Barron (1968), Domsch et al. (1980), and Gilman (1968).

Variations in the number of species (richness) and the number of isolates (density) were analyzed using one-way analysis of variance at the 0.05% level.

### Results and Conclusions

A total of 36 soil fungal species were identified from the rose bengal-streptomycin plates. As expected, the number of isolates significantly increased with an increase in the number of plates inoculated (table 1). In contrast, the total number of fungal species in any one

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sample peaked at 21 when 311 fungal colonies were counted (fig. 1). However, 21 does not differ significantly from the 17 fungal species isolated from 189 colonies. Thus, the minimum number of fungal colonies growing on rose bengal-streptomycin agar plates that would have to be isolated in order to begin to describe differences in the number of fungal species between

Table 1.—Fungal parameters associated with an undisturbed pinyon/juniper plant community in west-central New Mexico.

Number of plates	Number of isolates	Number of species
5	84d	14b
10	189c	17ab
15	250bc	19a
20	311b	21a
25	397a	20a

<sup>1</sup>Means within the same column followed by the same letter are not significantly different at the 0.05 level by Tukey's multiple range test.

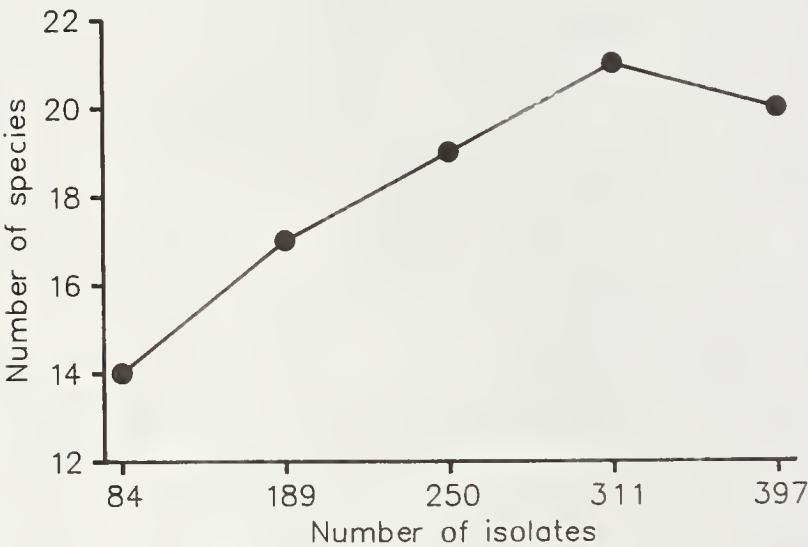


Figure 1.—Fungal species area curve from an undisturbed pinyon/juniper soil.

reclaimed areas from soil collected from an undisturbed pinyon/juniper plant community within the McKinley Coal Mine was 189.

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